National Toxicology Program
Toxicity Report Series
Number 49

NTP Technical Report on the Toxicity Studies of

1,1,2,2-Tetrachloroethane

(CAS No. 79-34-5)

Administered in Microcapsules in Feed to F344/N Rats and B6C3F$_1$ Mice

March 2004

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund (Superfund) by an interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (800-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Study Reports printed since 1991 appears at the end of this Toxicity Study Report.

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PEER REVIEW

The draft report on the toxicity studies of 1,1,2,2-tetrachloroethane was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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CONTENTS

SUMMARY

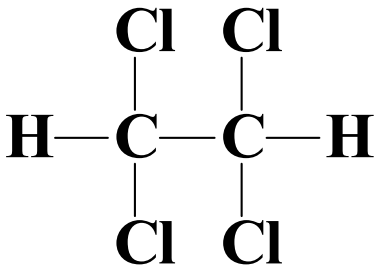
Background: 1,1,2,2-Tetrachloroethane was widely used in the production of solvents and pesticides. Its production ended in the 1990s, but it is a major component of waste sites. We studied the effects of 1,1,2,2-tetrachloroethane on male and female rats and mice to identify potential toxic hazards to humans.

Methods: Because 1,1,2,2-tetrachloroethane can evaporate easily, we enclosed it in starch microcapsules and placed them in the feed of rats and mice for 14 weeks. Male and female rats received up to 4,600 parts per million (ppm) 1,1,2,2-tetrachloroethane (equivalent to 0.46%) and mice received up to 9,100 ppm (0.91%). Control animals received empty starch microcapsules in their feed. Tissues from more than 40 sites were examined in all control and high-dose animals; tissues with lesions were examined in the lower exposure groups until no lesions were observed.

Results: Rats receiving 1,180 ppm or more 1,1,2,2-tetrachloroethane and mice receiving 2,300 ppm or more weighed less than the control animals. Male and female rats given 1,1,2,2-tetrachloroethane had pale and diseased livers and also had atrophy of the bone marrow and of the genital systems. Male and female mice given 1,1,2,2-tetrachloroethane had lesions of the liver and the bile duct.

Conclusion: We conclude that 1,1,2,2-tetrachloroethane at doses greater than 590 ppm in the feed was toxic to the liver of male and female rats. In mice, 1,1,2,2-tetrachloroethane was already known to cause cancer after long-term exposure. In these 14-week studies, 1,1,2,2-tetrachloroethane was toxic to the livers of male and female mice.

ABSTRACT



1,1,2,2-TETRACHLOROETHANE

CAS No. 79-34-5

Chemical Formula: $C_2H_2Cl_4$ Molecular Weight: 167.86

Synonyms: Acetylene tetrachloride; 1,1-dichloro-2,2-dichloroethane; *sym*-tetrachloroethane; TCE; 1,1,2,2-TCE; tetrachloroethane
Trade names: Acetosol, Bonoform, Boroform, Cellon

1,1,2,2-Tetrachloroethane is a solvent that was used in soil sterilization and as an ingredient in herbicides, insecticides, paints, varnishes, metal cleaners, and degreasers. Its production in the United States as an end-product ceased in the early 1990s. 1,1,2,2-Tetrachloroethane is currently used only as a chemical intermediate in the production of other chemicals. It was nominated for study because it was widely used and because it is found in hazardous waste sites and in surface water and groundwater. F344/N rats and B6C3F$_1$ mice were administered 1,1,2,2-tetrachloroethane (at least 99% pure) in microcapsules in the feed for 15 days or 14 weeks. Animals were evaluated for clinical pathology, reproductive system effects, and histopathology. Genetic toxicity studies were conducted *in vitro* in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, and Chinese hamster ovary cells and *in vivo* in *Drosophila melanogaster* and mouse peripheral blood erythrocytes.

In the 15-day studies, groups of five male and five female rats and mice were fed diets containing 3,325, 6,650, 13,300, 26,600, or 53,200 ppm microencapsulated 1,1,2,2-tetrachloroethane. Additional groups of five male and five female rats and mice served as untreated controls, receiving feed without microcapsules, or as vehicle controls, receiving feed with empty microcapsules. Exposure concentrations of 3,325, 6,650, and 13,300 ppm resulted in average daily doses of 300, 400, and 500 mg 1,1,2,2-tetrachloroethane per kilogram body weight to male and female rats. All rats and mice exposed to 53,200 ppm, all rats and male mice exposed to 26,600 ppm, and two male mice exposed to 13,300 ppm died

or were killed moribund before the end of the studies. The mean body weights of all exposed groups of rats and mice with survivors were significantly less than those of the vehicle controls, and all of these groups except 3,325 ppm male rats and female mice lost weight during the studies. Clinical findings included thinness and ruffled fur in rats and mice; 53,200 ppm rats and 26,600 and 53,200 ppm male mice were lethargic, while male mice in the lower exposure groups and exposed female mice (except the 53,200 ppm group) were hyperactive. Thymus weights of rats exposed to 6,650 or 13,300 ppm and all exposed groups of female mice were significantly less than those of the vehicle controls. Liver weights of male rats in the 13,300 ppm group were also significantly less than those of the vehicle controls.

At necropsy, thin carcasses were noted in all exposed groups of male rats, in female rats exposed to 13,300 ppm or greater, in male mice exposed to 6,650 or 13,300 ppm, and in female mice exposed to 13,300 or 26,600 ppm. In rats, hepatodiaphragmatic nodules were noted grossly in one untreated control female, one female exposed to 6,650 ppm, one male and one female exposed to 13,300 ppm, and two males and one female exposed to 26,600 ppm; mild or moderate centrilobular degeneration was observed microscopically in the exposed rats with liver nodules. Pale or mottled livers were noted in all groups of exposed male and female mice and correlated microscopically with hepatocellular degeneration; the severity of hepatocellular degeneration increased with increasing exposure concentration.

In the 14-week studies, groups of 10 male and 10 female rats were fed 268, 589, 1,180, 2,300, or 4,600 ppm microencapsulated 1,1,2,2-tetrachloroethane, and groups of 10 male and 10 female mice received 589, 1,120, 2,300, 4,550, or 9,100 ppm, which resulted in average daily doses of 20 to 320 mg/kg for male and female rats, 100 to 1,360 mg/kg for male mice, and 80 to 1,400 mg/kg for female mice. Additional groups of 10 male and 10 female rats and mice served as untreated and vehicle controls. Groups of 10 male and 10 female special study rats designated for hematology and clinical chemistry analyses on study days 5 and 21 received the same exposure concentrations as the core study rats. All core study animals survived to the end of the studies. The mean body weights of male and female rats exposed to 1,180 ppm or greater and male and female mice exposed to 2,300 ppm or greater were generally significantly less than those of the vehicle controls. Male and female rats in the 4,600 ppm groups lost weight during the study. Clinical findings of toxicity included thinness and pallor in all rats in the 2,300 and 4,600 ppm groups and thinness in mice exposed to 2,300 ppm or greater. Results of the functional observation battery indicated no exposure-related findings of neurotoxicity in rats or mice.

Results of the hematology and clinical chemistry analyses indicated that exposure of rats and mice to 1,1,2,2-tetrachloroethane induced a hepatic effect, as demonstrated by increases in serum alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, and 5′-nucleotidase activities and total bile acid concentrations. Decreases in serum concentrations of total protein and cholesterol could also have been related to a liver effect or may have been related to the nutritional status of the animals. There was evidence indicating an effect on the circulating

erythroid mass, characterized by a minimal to mild microcytic nonresponsive anemia, in exposed rats. Minimal decreases in platelet and lymphocyte counts also occurred in exposed rats.

The thymus weights of female rats exposed to 4,600 ppm were significantly less than those of the vehicle controls. The liver weights of male and female rats increased with increasing exposure concentration up to 1,180 ppm; at higher exposure concentrations, absolute liver weights decreased along with decreasing body weights, although relative liver weights remained increased. The liver weights of male mice in the 1,120 and 2,300 ppm groups and females in all exposed groups were significantly greater than those of the untreated and vehicle controls. Kidney weights of male mice exposed to 2,300 ppm or greater were significantly less than those of the vehicle controls.

Thin carcasses, pale livers, and/or liver foci were noted grossly in exposed male and female rats and mice in the 14-week studies; additionally, exposed male rats had small testes and seminal vesicles and exposed female rats had small or thin uteri. Pale kidneys were observed in one male mouse in each of the 4,550 and 9,100 ppm groups. Microscopic lesions of minimal to moderate average severity were observed in the liver of exposed male and female rats, and splenic lesions were observed in male and female rats administered 1,180 ppm or greater. Male and female rats in the 4,600 ppm groups and females in the 2,300 ppm group also had atrophy of the bone metaphysis and bone marrow, prostate gland, preputial gland, seminal vesicle, testicular germinal epithelium, uterus, and clitoral gland. The incidence of cytoplasmic alteration of the ovarian interstitial cells was significantly increased in female rats in the 4,600 ppm group.

Liver hepatocyte hypertrophy and necrosis, focal pigmentation, and bile duct hyperplasia were observed in exposed male and female mice in the 14-week study. Males also had increased incidences of preputial gland atrophy.

Results of reproductive tissue evaluations in the 14-week studies indicated decreased left cauda epididymis, left epididymis, and left testis (mice) weights and epididymal spermatozoal motility in exposed male rats and mice relative to the vehicle controls. Female rats in the 2,300 ppm group spent more time in diestrus and less time in proestrus, estrus, and metestrus than did vehicle control females. The estrous cycle of female mice in the 9,100 ppm group was longer than that of the vehicle controls.

1,1,2,2-Tetrachloroethane was negative for induction of mutations in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9 metabolic activation. It did not induce trifluorothymidine resistance in L5178Y mouse lymphoma cells with or without S9. In cytogenetic tests with cultured Chinese hamster ovary cells, 1,1,2,2-tetrachloroethane induced sister chromatid exchanges but not chromosomal aberrations in the presence and the absence of S9. No increases in the frequencies of sex-linked recessive lethal mutations were observed in germ cells of male *D. melanogaster* administered 1,1,2,2-tetrachloroethane via feeding or injection. Positive results were obtained

in the *in vivo* peripheral blood micronucleus test in mice in the 14-week feed study; significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in males and females.